

Th₁/Th₂ Cytokine Profile and Its Diagnostic Value in *Mycoplasma pneumoniae* Pneumonia

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Abstract

Background: The levels of Th₁/Th₂ cytokine can alter in pathogenic infection in children with pneumonia.

Objectives: To evaluate Th₁/Th₂ cytokine profile and its diagnostic value in *M. pneumoniae* pneumonia in children.

Patients and Methods: Children with *M. pneumoniae* mono-infection and 30 healthy children were tested with cytokines assay. We used real time PCR to detect *M. pneumoniae* in children with pneumonia.

Results: *M. pneumoniae* test was positive in 2188 (16.62%) out of 13161 pneumonia children. Children aged 5 - 9 years had the highest rate and summer was a season with high rate of *M. pneumoniae* incidence in Zhejiang province. During the course of study, in 526 pneumonia children with *M. pneumoniae* mono-infection and 30 healthy children cytokines assay was performed. IL-2 level of *M. pneumoniae* pneumonia children was lower than that of healthy children (median levels, pg/mL: IL-2: 3.2 vs. 5.7, P = 0.00), while IL-4, IL-10 and IFN- γ were higher than in healthy children (median levels, pg/mL: IL-4: 3.2 vs. 1.5, P = 0.00; IL-10: 5.6 vs. 2.5, P = 0.001; IFN- γ : 20.4 vs. 4.8, P = 0.001).

Conclusions: IL-2 decreases and IL-4, IL-10 and IFN- γ increase in children with *M. pneumoniae* pneumonia, which has a promising prospect in diagnosis of this disease in clinical practice.

Keywords: Cytokines, Pneumonia, Children, *M. pneumoniae*

1. Background

M. pneumoniae is one of the major pathogens of community-acquired pneumonia in children all over the world (1-3). *M. pneumoniae* easily attaches to ciliated epithelial cells of the respiratory tract and causes damage (4, 5). For diagnosis of *M. pneumoniae*, culture and serological diagnosis are common methods in clinical practice, but insensitivity, time-consuming or cross-reactions limit their application (6-10). Recently, PCR method was used to directly detect *M. pneumoniae* DNA, which has high specificity and sensitivity in clinical detection of *M. pneumoniae* (11, 12). But in clinical practice, co-infections are common in children with pneumonia and direct detection of *M. pneumoniae* is not efficient in distinguishing it from pneumonia (8). Our previous study suggested that Th₁/Th₂ balance plays a significant part in anti-infectious immunity and Th₁/Th₂ cytokines are useful biomarkers in diagnosis and treatment of bacterial and viral infection (13-15).

2. Objectives

In this study, we wanted to confirm the value of Th₁/Th₂ cytokines in diagnosis and treatment of *M. pneumoniae* pneumonia.

3. Patients and Methods

Subjects: From May 2012 to June 2014, a total of 13161 throat swab specimens were collected. The age of the 13161 patients ranged from 3 months to 10 years. All these children had been primarily diagnosed with pneumonia (16) and had received no clinical treatment.

Detection of *M. pneumoniae*: *M. pneumoniae* PCR kit (Daan Gen Co., Ltd. Guangzhou, China) was used in DNA extraction and *M. pneumoniae* DNA detection (4). Amplification and data analysis were carried out with an applied biosystems 7500 real-time PCR system (Applied Biosystems, Inc., CA, USA) under this condition: 93°C for 2 minutes and 40 cycles of 93°C for 45 seconds and 55°C for 60 seconds. Specific tests were used to detect other pathogens, such as immunofluorescence to detect respiratory viruses including adenovirus, human metapneumovirus, respiratory syncytial virus, parainfluenza virus and influenza virus; blood and sputum culture for bacteria; and real-time PCR to detect human cytomegalovirus, Epstein-Barr virus, *Chlamydia pneumoniae*, *Ureaplasma urealyticum* and *Chlamydia trachomatis*. If any of these assays was positive, the patient was excluded from the study.

Cytokine determination: The protocol of cytokine measurement was reported in our previous study (13) as fol-

lows: The clotted blood samples were centrifuged at 1,000 g, 20°C for 20 minutes. After that, the supernatant was collected and the levels of Th₁/Th₂ cytokines by 320 flow cytometry detected. IL-2, IL-4, IL-6, IL-10, TNF- α and IFN- γ were detected quantitatively by CBA kit-BDTM CBA Human Th₁/Th₂ Cytokine Kit II (BD Biosciences, San Jose, CA). After collecting the sample data on a FACScalibur™ flow cytometer (Becton Dickinson, San Jose, CA, USA), we used the BD CBA Software (BD Biosciences, San Jose, CA, USA) to display the results in tabular and graphical format. Then we established the standard curve for each reagent. 1.0 pg/mL was the lowest detection limit for these six cytokines, while the highest was 5,000 pg/mL.

Statistical analysis: We used χ^2 or Fisher's exact test and Mann-Whitney U test in SPSS Statistics 19.0 software. It was considered that $P < 0.05$ is statistically significant. We used receiver operating characteristic (ROC) curve to estimate the value of cytokines in diagnosing *M. pneumoniae* pneumonia by SPSS Statistics 19.0 software.

4. Results

Patients' characteristics: Between May 2012 and June 2014, 13161 throat swab samples were collected and tested for *M. pneumoniae*, including 1353 from outpatients and 11808 from hospitalized children. 8082 samples were from boys and 5079 from girls, yielding a male-to-female ratio of 1.59:1. 2188 were tested positive for *M. pneumoniae*, with a positive rate of 16.62%. Among 2188 *M. pneumoniae* positive samples, 1277 were from boys and 911 from girls, giving positive rates of 15.80% in boys and 17.93% in girls.

As shown in Figure 1, positive rate for *M. pneumoniae* infection was at the peak in children aged 5-9 years (42.46% - 46.92%, compared with other group, $P < 0.01$). It steadily declined with increasing or decreasing age. Among age groups, children younger than 1 year had the lowest (7.39%) positive rate, compared with the other group ($P < 0.01$). *M. pneumoniae* infection occurred all year round, the monthly positive rates for *M. pneumoniae* infection ranged from 7.65% to 27.35%, with a peak in June and August (compared with other group $P < 0.01$), and steadily declined in the previous and the following months (Figure 2). Co-infections were found in 1662 (75.96%) *M. pneumoniae* positive children, which were higher than in mono-infection children.

Inflammatory cytokine levels: To evaluate the levels of these six cytokines in healthy children and children with *M. pneumoniae* pneumonia. 526 patients with single *M. pneumoniae* infection were used as *M. pneumoniae* pneumonia group and 30 healthy children acted as the control group. As shown in Table 1, comparisons between *M. pneumoniae* infection group and normal control group revealed no difference of inflammatory cytokine (IL-6 and TNF- α) levels between the two groups (median levels, pg/mL: IL-6: 14.27 vs. 4.54, $P = 0.057$; TNF- α : 3.56 vs. 2.21, $P = 0.182$). The level of IL-2 was significantly lower in serum from *M. pneumoniae* pneumonia patients than in serum

from normal controls (median levels, pg/mL: IL-2: 3.19 vs. 5.72, $P = 0.00$), while the levels of IL-4, IL-10 and IFN- γ in *M. pneumoniae* pneumonia patients were significantly higher than in the normal controls (median levels, pg/mL: IL-4: 3.23 vs. 1.46, $P = 0.00$; IL-10: 5.56 vs. 2.53, $P = 0.001$; IFN- γ : 20.35 vs. 4.83, $P = 0.001$).

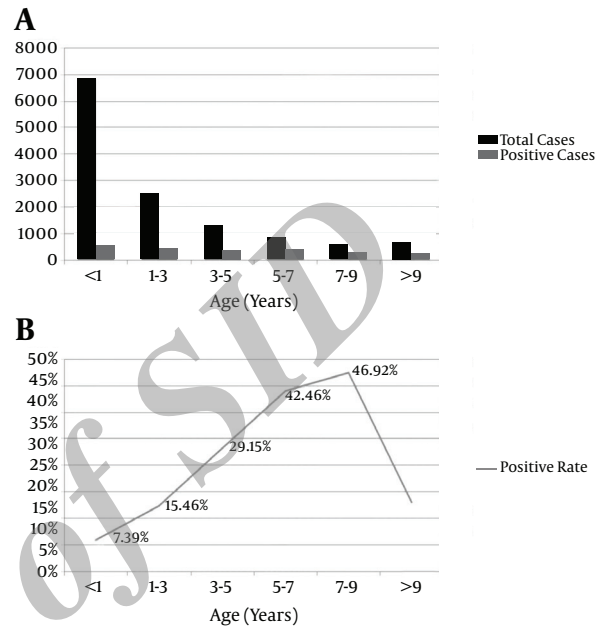


Figure 1. Age Distribution of *M. Pneumoniae* Pneumonia in Children Younger Than 14 Years

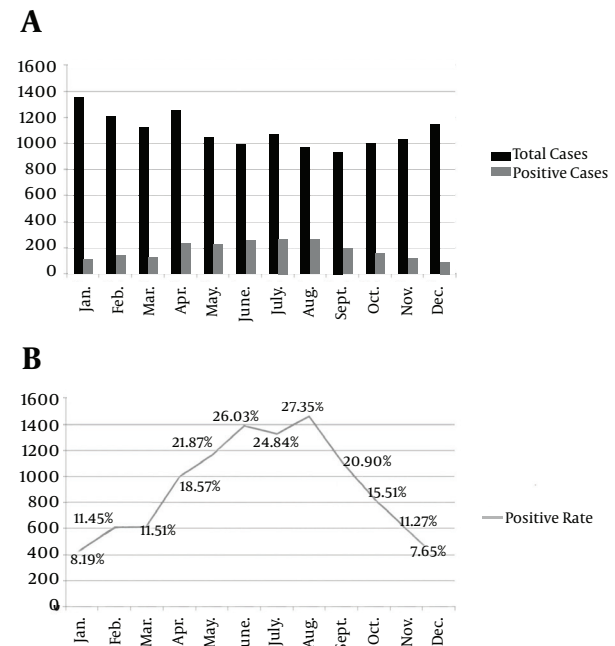


Figure 2. Monthly Distribution of *M. Pneumoniae* Pneumonia in Children Younger Than 14 Years.

Table 1. Serum Cytokine Levels in the Control Group and *M. pneumoniae* Pneumonia Group

Parameters	MPP (n = 526)	Control (n = 30)	P Value
IL-2	3.2 (1.0 - 7.0)	5.7 (2.7 - 7.8)	< .001
IL-4	3.3 (1.3 - 6.3)	1.5 (1.0 - 2.1)	< .001
IL-6	54.3 (1.0 - 1459.0)	4.5 (1.2 - 8.5)	.057
IL-10	5.6 (1.0 - 49.9)	2.5 (1.3 - 3.7)	.001
TNF- α	3.6 (1.0 - 133.8)	2.2 (1.3 - 3.1)	.182
IFN- γ	20.4 (3.0 - 158.5)	4.8 (3.3 - 7.8)	.001

Abbreviations: Control, healthy children group; MPP, *M. pneumoniae* pneumonia group.

Table 2. ROC Curve for Diagnostic Value of Cytokines for *Mycoplasma pneumoniae* Pneumonia

Cytokines	AUC	95% CI	Sensitivity, %	Specificity, %
IL-2	.922	.878 - .966	88.1	84.8
IL-4	.954	.928 - .979	81.9	100
IL-6	.729	.678 - .780	62.8	95.3
IL-10	.819	.758 - .880	86.3	82.1
TNF- α	.702	.629 - .775	59.3	78.8
IFN- γ	.928	.892 - .963	80.2	93.9

ROC-analysis: To confirm value of inflammatory cytokines in diagnosis of *M. pneumoniae* pneumonia, we used ROC-analysis to evaluate the abilities of the six cytokines in identifying the possibility of *M. pneumoniae* pneumonia. The AUCs were 0.922 (95% CI, 0.878 to 0.966), 0.954 (95% CI, 0.928 to 0.979), 0.819 (95% CI, 0.758 to 0.880) and 0.928 (95% CI, 0.892 - 0.963) for IL-2 (lower than 4.5pg/mL), IL-4 (greater than 2.5 pg/mL), IL-10 (greater than 3.0 pg/mL) and IFN- γ (greater than 5.5 pg/mL) respectively, with sensitivity and specificity above 80% (Table 2). These results indicated that IL-2, IL-4, IL-10 and IFN- γ could be effective biomarkers to identify *M. pneumoniae* pneumonia.

5. Discussion

M. pneumoniae pneumonia is common in children, in clinical practice it is important to accurately diagnose *M. pneumoniae* infection (1, 2). In this article, we used real time PCR to detect *M. pneumoniae* in throat swab samples. Our results showed that positive rate for *M. pneumoniae* was highest among children aged 5 - 9 years and summer was the *M. pneumoniae* season in China. There are common methods to detect *M. pneumoniae* in clinical practice, such as PCR, culture and serological test. Interestingly, co-infections were found in 1662 (75.96%) of the *M. pneumoniae* positive cases. It was difficult to estimate the true association between the clinical manifestations and *M. pneumoniae* infection (8). So, in this study only children with *M. pneumoniae* infection were evaluated with Th₁/Th₂ cytokines level. Our previous study has shown that the use

of biomarkers to diagnose bacterial infection in children were IL-2, IL-6 and IL-10 (14). In contrast to the normal controls, the levels of IL-2, IL-4, IL-10 and IFN- γ in children with *M. pneumoniae* pneumonia had significantly changed and had high sensitivity and specificity. Among them, IL-2 can fight against infection and plays a unique role in generating and maintaining regulatory T-cells (15). Therefore, IL-2 often decreases at the time of and after infection. After *M. pneumoniae* infection, changing IL-2 level was reported in previous studies with controversial results (16, 17). Our study supports findings indicating that IL-2 level decreases after *M. pneumoniae* infection in children. IL-10 has pleiotropy, which can limit antigen-presenting cell function and primarily inhibits antigen-presenting cells from releasing chemokines and proinflammatory cytokines (13, 14). Furthermore, it also can directly suppress T-cell proliferation, function and cytokine production to limit inflammation (14). IL-10 increases and plays an important role as anti-inflammation agent (18, 19). This is the underlying mechanism of regulating IL-2 and IL-10 during *M. pneumoniae* infection in children. Cytokines which are produced by Th₁ cells can be blocked by IL-4 produced by Th₂ cells (18). IL-4 also plays a vital role of switching to IgE which induces allergic diseases (20, 21). Additionally, increase of IFN- γ during *M. pneumoniae* infection was also shown in a previous report (22).

M. pneumoniae is a most common pathogen which causes pneumonia in children in Hangzhou, China. Our study suggests that the effective biomarkers which can be used to diagnose whether children with pneumonia are afflicted by *M. pneumoniae* are IL-2, IL-4, IL-10 and IFN- γ .

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